A COMPARATIVE STUDY OF FLUORESCENT STAINING AND ZIEHL NEELSEN’S STAINING FOR DETECTION OF ACID FAST BACILLI IN SPUTUM IN A TERTIARY CARE HOSPITAL IN MANGALORE.

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ABSTRACT:

Introduction: Our country has a high burden of tuberculosis (TB) with a prevalence of 211 cases per 100,000 populations. With limited resources, the diagnosis of tuberculosis (TB) relies primarily on smear microscopy for Acid Fast Bacilli (AFB).

Material and methods: The study was conducted over a period of 6 months. Sputum samples were collected and processed for AFB detection by Ziehl Neelson staining and Fluorochrome method.

Results: A total of 861 samples were included of which by fluorescent staining 114 (13.24%) samples were positive and by Ziehl–Neelsen’s staining 89 (10.33%) samples were positive. Fluorescent staining method detected twenty five more sputum smear acid fast bacilli than Ziehl–Neelsen’s staining method.

Conclusions: Our study showed that the fluorescent staining method has better sensitivity than Ziehl-Neelsen’s in detection of acid fast bacilli. Fluorescent staining detects acid fast bacilli in low densities. Fluorescent method is more reliable and easy whenever dealing with large number of samples.

KEYWORDS: Fluorescent Staining, Acid Fast Bacilli

INTRODUCTION

Tuberculosis (TB), the captain of death, has been and is still lingering as a major threat to human health. It is estimated that nearly one billion people will be infected with tuberculosis (TB), and 200 million develop the disease and 35 million will die from tuberculosis (TB) during 2000-20201. Our country has a high burden of tuberculosis (TB) with a prevalence of 211 cases per 100,000 population and 171 incident cases during 20132. With limited resources, the diagnosis of tuberculosis (TB) relies primarily on smear microscopy for Acid Fast Bacilli (AFB). The techniques are simple and detect those cases of tuberculosis, which are infectious. Sputum microscopy is also useful to assess the success of treatment and to affirm whether patient is cured or not3. The principle of staining is that Mycobacteria retain the primary stain even after exposure to decolorizing agent, hence called acid fast.
Ziehl – Neelsen stain still remains the standard method used throughout the world for detection of pulmonary tuberculosis, but it is considered less sensitive compared to fluorescent stain. Basically the same technique is utilized by fluorescence staining as Ziehl-Neelsen staining barring that carbol fuchsin is replaced by a fluorescent dye. Most important advantage of fluorescence technique is that lower magnification is enough to scan the field and time needed to examine the same area is reduced by five times. The present study was conducted to evaluate the efficiency of smear microscopy to detect acid fast bacilli (AFB) stained by Ziehl-Neelsen method and Fluorescent method after sputum concentration by Petroff's method.

MATERIALS AND METHODS

The present study was conducted at Department of Microbiology, Yenepoya Medical College. Deralakatte, Mangalore during the period of 1st November 2014 to 30th April 2015. A total of 861 sputum samples were randomly collected from patients attending Yenepoya Medical college hospital, with history of cough for more than two weeks with sputum, fever, night sweats, haemoptysis and radiological evidence of tuberculosis. A single sputum sample was collected for each patient on the spot in clean sterile leak proof wide mouth containers. The processing of samples were carried out in biosafety cabinet. All sputum samples were concentrated by Petroff's method.

Preparation of Ziehl-Neelsen (Z-N) smear: Z-N smear was done as per Revised National Tuberculosis Control Programme (RNTCP) guidelines. Smear was heat fixed and flooded with 1% carbol fuchsin stain. Heated from below until vapour just started and stain was allowed to stand for five minutes and then washed with running tap water. 25% sulphuric acid was applied for three minutes and washed again in running tap water. Smear was counter stained with 0.1% methylene blue for one minute. Smear was air dried and observed under microscope using oil immersion objectives. Presence of slender pink coloured bacilli in blue background indicates positivity of Z-N staining. Smear is graded as per RNTCP criteria as 3+, 2+, 1+, scanty or negative.

Preparation of Fluorescent smear - The heat fixed smear was flooded with Auramine –O for twenty minutes .Then rinsed well with running tap water taking care so as not to wash away smear. Decolourise with acid alcohol for three minutes and rinse in running tap water. Quench with 0.5% potassium permanganate, air dried and examined under high power. Acid fast bacilli (AFB) typically fluoresce as golden, slender rod shaped structure. Smear is graded as per RNTCP criteria.

RESULTS

A total of 861 sputum samples obtained from patients were processed by both fluorescent staining and Ziehl – Neelsen’s staining. By fluorescent staining 114 (13.24%) samples were positive and 747 (86.75%) samples were negative for acid fast bacilli. In Ziehl-Neelsen’s staining 89 (10.33%) samples were positive and 772 (89.66%) were negative for acid fast bacilli (Table 1).

Fluorescent staining method detected twenty five more sputum smear acid fast bacilli than Ziehl –Neelsen’s staining method (Table 2).

DISCUSSION

Since radiometric and molecular methods for diagnosis of tuberculosis is very costly, time consuming and need expertise, in developing country like India with limited settings of well-equipped laboratory settings sputum microscopy mainly Ziehl-Neelsen staining is done in designated microscopic centres (DMC) and government based health care providers coming under Revised National Tuberculosis Control programme (RNTCP). Sputum microscopy is fastest, cheapest and reliable. Now fluorescent microscopy is added in many DMC’s as it is more sensitive and rapid result and can be used in field areas. The comparison of the fluorescent method with conventional Ziehl-Neelsen method was implicated to improve the smear positivity for the detection of acid fast bacilli. The use of fluorescence stains such as Auramine-O is recommended because of its increase sensitivity and ease to interpret compared with the Ziehl-Neelsen method. The present study was an attempt to compare the ability of fluorescent staining method and Ziehl-Neelsen’s staining in detecting acid fast bacilli mainly when the bacterial load is less in sputum samples. The present study showed that the sensitivity of Auramine-O staining was much better than Ziehl-Neelsen’s staining. The results of the present study agrees with the results of other studies. Use of fluorescent microscopy significantly increases the diagnostic value of the smear, particularly when there are low density of bacilli, which may escape detection on Ziel-Neelsen’s staining. This is particularly true in case of scanty bacilli in sputum, which was missed by Ziehl-Neelsen’s staining. Due to the presence of unsaponifiable wax substance in the cell wall of tubercle bacilli Ziehl-Neelsen method of staining method needs heat application to microscopic slide for the penetration of dye into the cell wall. But fluorescent staining doesn’t need heating. This makes it economical in both time and expense and recommended for laboratories handling large number of samples.

CONCLUSION:

This study showed that the fluorescent staining method has better sensitivity then Ziehl-Neelsen in detection of acid fast bacilli. Fluorescent staining detects acid fast bacilli in low densities. Whenever sputum sample contains
scanty bacteria fluorescent method detects them easily. It is quite economical in terms of time and expense. Fluorescent method is more reliable and easy whenever dealing with large number of samples.

Table 1: Result of smear examination by Ziehl-Neelsen’s staining and Fluorescent staining methods

<table>
<thead>
<tr>
<th></th>
<th>Z-N staining</th>
<th>Fluorescent staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>772 (89.66%)</td>
<td>747 (86.75%)</td>
</tr>
<tr>
<td>Positive</td>
<td>89 (10.33%)</td>
<td>114 (13.24%)</td>
</tr>
<tr>
<td>Total</td>
<td>861(100%)</td>
<td>861(100%)</td>
</tr>
</tbody>
</table>

Table 2: Performance of Ziehl-Neelsen’s staining and Fluorescent staining

<table>
<thead>
<tr>
<th></th>
<th>Z-N Positive</th>
<th>Fluorescent Positive</th>
<th>Z-N Negative</th>
<th>Fluorescent Negative</th>
<th>Total AFB Positive</th>
<th>Total Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>89 (10.33%)</td>
<td>114 (13.24%)</td>
<td>25 (2.90%)</td>
<td>0</td>
<td>114</td>
<td>861</td>
</tr>
</tbody>
</table>

REFERENCES: